XENORHABDUS SP. GENOME SEQUENCES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part (CIP) application of U.S. Non-provisional Application Ser. No. 12/385,507, filed Apr. 9, 2009, which is a continuation of U.S. Non-provisional application Ser. No. 12/289,606, filed Oct. 30, 2008, which is a continuation of U.S. Non-provisional application Ser. No. 09/897,516, filed Jun. 29, 2001, which claims priority of U.S. Provisional Application 60/215,161, filed Jun. 30, 2000. Each of the aforementioned US applications is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to genomic nucleic acid sequences from *Xenorhabdus* sp., in particular, and to *Photorhabdus* sp., and includes nucleic acid molecules present in both coding and in non-coding regions. Nucleic acid sequences that encode proteins and/or enzymes and homologues and fragments thereof are encompassed by the invention including but not limited to insect inhibitory proteins, proteins capable of conferring antibiotic resistance, microbial inhibitory proteins including bactericidal, bacteriostatic, fungicidal, and fungistatic proteins, proteins capable of conferring resistance to heavy metals or other toxic compositions, proteins and compositions capable of conferring pharmaceutical advantages such as antineoplastic, acaricidal, anti-inflammatory and anti-ulcerogenic properties, polyketide synthases, transposons and mobile genetic elements and their corresponding transposases, excisases and integrases, phage and phage particle proteins, other useful *Xenorhabdus, Photorhabdus, Serratia, Yersinia, Salmonella, E. coli*, and *Erwinia* sp. protein homologues, ribosomal RNA (rRNA), and transfer RNA (tRNA). In addition, proteins and fragments thereof so encoded and antibodies capable of binding the proteins are encompassed by the present invention. The invention also relates to methods of using the disclosed nucleic acid molecules, proteins, fragments of proteins, and antibodies, for example, for gene identification and analysis, preparation of constructs, transformation of cells with nucleotide compositions disclosed herein to produce *Xenorhabdus* proteins or fragments thereof, in particular novel insect inhibitory, bactericidal, fungicidal and nematicidal proteins.

BACKGROUND OF THE INVENTION

[0003] *Xenorhabdus* sp. and *Photorhabdus* sp. strains have previously been shown to produce an array of extracellular proteins and small molecules or secondary metabolites having specialized functions. Among the more commercially interesting are proteins and small molecules having antibiotic properties or proteins which exhibit insect inhibitory activity. A small number of insect inhibitory proteins have previously been identified from these bacteria, symbionts of insect-parasitic nematodes. In view of the biotechnology methods which are now available, such proteins and compositions have great potential for use as biologically safe and effective pest control agents. Unlike chemical pesticide compositions, these proteins have no effect upon the environment in general, can be targeted to direct their effect primarily upon target insect species, and have no effect on non-target species. These proteins are comparable in nature to *Bacillus thuringiensis* (BT) proteins, which are the most widely used biological insect pest control agents derived from various strains of *Bacillus thuringiensis*. BT compositions have been in commercial use for more than twenty years as topically applied insect control agents and more recently genes encoding various BT proteins have been expressed in transgenic plants, and in particular in agronomically important crops such as soybean, corn, wheat, rice, and cotton. However, one issue related to the use of BT proteins is resistance management. The concern is that target insect pests feeding on a plant expressing a single BT protein that is generally effective against that pest species will develop resistance to the protein in some calculable period of time. The answer to this problem has been to include in the plant another BT protein also toxic to the same target pest species. The idea is similar in nature to bacterial resistance management, in that the development of resistance to either of the BT proteins will be delayed because pest will not produce progeny that are resistant to either of the BT proteins, in particular if the two proteins that are expressed in the plant have different modes of action or bind different receptors in the insect midgut. Unfortunately, BT proteins are highly related and often it is difficult to distinguish whether two BT proteins toxic to the same insect species have different modes of action. Thus, even though a great variety of BT proteins have been identified, characterized and categorized into distinct classes of proteins, all appear to act in a very similar fashion. Therefore, a different resistance management strategy which takes advantage of insect inhibitory proteins derived from distinct microbial sources other than *Bacillus thuringiensis* would be desirable. Insect inhibitory proteins isolated from *Xenorhabdus* and *Photorhabdus* species of bacteria seem to have all the prerequisites for the delivery of novel genes for transgenic expression of insect pest inhibiting proteins to provide pest resistance to plants, either alone or in combination with *Bacillus thuringiensis* insecticidal crystal proteins.

[0004] *Xenorhabdus* sp. is a Gram-negative bacterium, member of the family of Enterobacteriaceae, and symbiotically associated with nematodes of the genus *Steinernema*. The nematode-bacterial complex can be characterized as an obligate and lethal parasitic relationship, specializing in parasitizing and proliferating in soil insect larvae. Infective, non-feeding stages of these nematodes live in soil and carry their nematode-genus-specific symbiotic bacteria in the gut. It is believed that the nematodes actively search for the appropriate insect host, invade the insect larvae through natural openings or lesions in the cuticle and, once inside the hemolymphe, release their symbiotic bacteria. The nematode-bacterial complex secretes a variety of highly efficient extracellular metabolites and proteins exhibiting insectic inhibitory, bactericidal, fungicidal and nematicidal properties to secure the larval mass as a source of nutrition. An array of extracellular enzymes such as lipases, phospholipases, proteases, nucleases as well as several broad spectrum antibiotics, and antifungal and nematicidal compositions are also secreted (Boemare & Akhurst, J. Gen. Microbiol. 134: 751-761 (1988); Li et al., Can. J. Microbiol. 43(8):770-773 (1997); McInerney et al., J. Nat. Prod. 54(3):774-84 (1991); McInerney et al., J. Nat. Prod. 54(3):785-95 (1991); Sundar and Chang, J. Gen. Microbiol. 139 (Pt 12):3139-48 (1993)). It has been discovered that some compounds secreted by *Xenorhabdus* exhibit anti-neoplastic (U.S. Pat. No. 5,827, 872), acaricidal, anti-inflammatory and anti-ulcerogenic properties (U.S. Pat. No. 4,837,222). U.S. Pat. No. 6,048,838